



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> A01N 65/00, 35/02, 37/18 A01N 43/22, 31/02, A61K 7/06 A61K 7/48, 31/35	<b>A1</b>	<b>(11) International Publication Number:</b> WO 91/08670 <b>(43) International Publication Date:</b> 27 June 1991 (27.06.91)
<b>(21) International Application Number:</b> PCT/AU90/00586 <b>(22) International Filing Date:</b> 11 December 1990 (11.12.90) <b>(30) Priority data:</b> PJ 7775 11 December 1989 (11.12.89) AU PJ 8651 16 February 1990 (16.02.90) AU <b>(71)(72) Applicant and Inventor:</b> GUERRINI, Vincent, Henry [AU/AU]; 173 Chatswood Road, Daisy Hill, QLD 4128 (AU). <b>(74) Agent:</b> HALFORD, Graham, William; Halford & Co., 49-51 York Street, Sydney, NSW 2000 (AU). <b>(81) Designated States:</b> AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.		<b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PESTICIDAL COMPOSITION COMPRISING AZADIRACTIN, DIALKYL TOLUAMIDE AND OIL (GERANIOL AND/OR CITRONELLAL)  <b>(57) Abstract</b>  Pesticidal compositions comprising synergistic combinations of azadirachtin, diethyltoluamide and a volatile oil such as oil of citronella. The compositions are suitable for direct application to the skin, coat of feathers of an animal or bird to prevent infestation by pests such as insects and arachnids.		

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PESTICIDAL COMPOSITION COMPRISING AZADIRACHTIN, DIETHYLTOLUAMIDE AND OIL (GERANIOL AND/OR CITRONELLAL)

The present invention relates to pesticidal compositions, and to methods of killing or repelling pests. In particular, the invention relates to synergistic mixtures of pesticidal compounds.

Diethyltoluamide (DEET) is a known pesticidal compound having low toxicity to animals and is used in insect repellent compositions such as Aeroguard (Registered Trade Mark). Oil of citronella is a natural oil containing geraniol and citronella with minor amounts of camphene, dipentene, linalool and borneol, which is extracted from fresh grass of Cymbopogon (Andropogon) nardus. Oil of citronella is also known as a pesticide with low toxicity to animals. However, DEET and oil of citronella individually are not effective in blocking the development of parasites, ectoparasites and insects except in high doses.

In pending Australian Patent Application No. 60185/90, the present Applicant discloses a synergy which exists between the known pesticidal agents diethyltoluamide (DEET) and oil of citronella. The synergy between these agents is particularly effective in blocking the development of larvae, and the mechanism of action is believed to relate to the synthesis of chitin.

In pending Australian Patent Application Nos. 75714/87, 11220/88, 11221/88 and 26320/88, the present Applicant describes the use of azadirachtin-rich extracts of neem seeds and other meliaceous trees to inhibit pests such as blowfly larvae, lice and fleas. Azadirachtin-rich extracts from neem seeds have been used in Indian folk medicine for thousand years and this, together with recent laboratory toxicological tests, has shown azadirachtin to be of very low toxicity to vertebrates.

SUBSTITUTE SHEET

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It is an object of the present invention to provide a new pesticidal composition in which the components exhibit true synergy, and to provide a new method of killing or repelling pests in which synergy between pesticidal compounds allows the use of lower doses.

The present invention thus provides a pesticidal composition comprising dialkyltoluamide, azadirachtin and an oil containing geraniol and/or citronellal.

The alkyl moieties in the dialkyltoluamide are preferably C<sub>1</sub> - C<sub>4</sub> alkyl, more preferably ethyl. In a most preferred embodiment, the dialkyltoluamide compound is N,N-diethyl-m-toluamide.

The oil is preferably oil of citronella, but may be any oil containing genaniol and/or citronellal, such as the oils of lemon grass, lemon, rose, lavender and penny royal. Alternatively, any volatile oils with pesticidal or insect repelling properties may be used.

Preferably, the pesticidal composition comprises from 1 to 35% by volume of oil of citronella, from 2 to 30g per litre Azadirachtin and from 30 to 200g per litre DEET. The composition may also comprise di-n-propyl isochinchomerate, N-octyl bicycloheptene dicarboximide, and/or other substances with pesticidal or insect repelling properties.

In order to prevent rapid oxidation of the azadirachtin, the carrier is preferably non-aqueous, more preferably alcoholic.

The invention also relates to a method of killing or repelling pests comprising applying dialkyltoluamide, azadirachtin and an oil containing geraniol and/or citronellal to the pests or their surroundings. The

dialkyltoluamide, azadirachtin and the oil may be applied together or sequentially.

5 Further preferred embodiments of the invention shall become apparent from reference to the Examples.

Example

10 One litre of a liquid pesticidal/insect repelling composition may be made by mixing the following:

Oil of Citronella	10 to 350ml
Lanolin	0.1 to 4g
Fatty Acid (e.g. Oleic acid)	5 to 100ml
15 Meliaceous extracts containing	
5% azadirachtin	40 to 600ml
or Pure azadirachtin	2 to 30g
Triclosan	0.17 to 1.16
N,N, Diethyl-M-Toluamide	30 to 200g
20 Di-n-propyl Isochinchomerate	6 to 46g
N-octyl bicycloheptene	1.5 to 10.5g
Alcohol (e.g. ethanol)	Remainder

Comparative Examples

25 The following liquids were mixed in different combinations to produce the preparations used in comparative Examples 1 to 4:

30 (a) Oil of citronella ("CIT").

(b) Aerogard Tropical Strength ("ATS") - containing 190g/kg DEET, 43.5g/kg di-N-propyl isochinchomerate, 10g/kg N-octyl bicycloheptene dicarboximide, and  
35 1.1g/kg Triclosan (a preservative) in a solvent comprising alcohol and lemon grass oil.

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(c) Aerogard Normal Strength ("ANS") - containing 96g/kg ethyl hexane diol (EHD), 27.2g/kg di-N-propyl isochinchomate, and 18g/kg N-octyl bicycloheptene dicarboximide in a solvent comprising alcohol and lemon grass oil.

(d) Alcoholic Neem extract ("Neem") containing 6% by weight azadirachtin.

(e) Alcoholic spirit ("ALC") comprising 96% ethanol.

Comparative Example 1 - three liquid preparations were made up by mixing components (a) to (e) set out above in the proportions set out in Table 1A.

TABLE 1A

Preparation No.	Proportion (Volume %)				
	CIT	ATS	ANS	NEEM	ALC
1-1	33.3	33.3	-	16.7	16.7
1-2	33.3	-	33.3	16.7	16.7
1-3	33.3	33.3	-	-	33.3

Preparations 1-1, 1-2 and 1-3 were used to treat respective groups of six greyhound dogs. Each treatment consisted of a single application of the preparation by spraying 40-50ml on each dog and rubbing this into the skin and fur. Each dog's kennel was sprayed with 40ml per square metre of the respective preparation.

Table 1B shows the mean number of fleas counted on

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each group before treatment (Day 0), and at 2, 8 and 17 days after treatment. The percentage increase or decrease in the number of fleas is given in parentheses.

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TABLE 1B

Preparation	Mean No. of Fleas			
	Day 0	Day 2	Day 8	Day 17
1-1	8.2	0.5(-94%)	2.3(-71%)	4.5(-45%)
1-2	10.0	4.2(-59%)	9.2(-8%)	7.8(-22%)
1-3	11.2	2.5(-78%)	5.2(-54%)	8.3(-25%)
Untreated	4.2	7.8(+88%)	9.3(+124%)	9.8(+136%)

As can be seen in Table 1B, a single application of preparation 1-1 containing Oil of Citronella, DEET and Azadirachtin reduced the flea population on the dogs by 94% by Day 2, and resulted in a reduction in the flea population of 45% after 17 days. In contrast, preparation 1-2 in which the DEET in preparation 1-1 was substituted by EHD, and preparation 1-3 which did not include azadirachtin, reduced the flea population after 17 days by only 22% and 25% respectively.

Comparative Example 2 - two liquid preparations were made up by mixing components (a) to (e) in the proportions set out in Table 2A.

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TABLE 2A

Preparation No.	Proportion (Volume %)				
	CIT	ATS	ANS	NEEM	ALC
2-1	33.3	33.3	-	16.7	16.7
2-2	-	-	-	50	50

Preparations 2-1 and 2-2 were used to treat respective groups of eight greyhound dogs. Each treatment consisted of a single application of the preparation by spraying 40-50ml on each dog and rubbing this into the skin and fur. Each dog's kennel was sprayed with 40ml per square metre of the respective preparation.

Table 2B shows the mean number of fleas counted on each group before treatment (Day 0), and at 2, 6 and 13 days after treatment. The percentage increase or decrease in the number of fleas is given in parentheses.

TABLE 2B

Preparation No.	Mean No. of Fleas			
	Day 0	Day 2	Day 6	Day 13
2-1	11.4	0.9(-92%)	2.3(-80%)	3.8(-57%)
2-2	9.9	4.8(-51%)	7.8(-21%)	8.8(-9%)
Untreated	4.6	6.0(+30%)	10.8(+146%)	12.3(+130%)



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As can be seen in Table 2B, a single application of preparation 2-1 according to the invention reduced the flea population by 92% by Day 2, with a reduction of 57% after 13 days. In contrast, preparation 2-2 which did not contain DEET or oil of citronella reduced the flea population after 13 days by only 9%.

Comparative Example 3 - eight liquid preparations were made up by mixing components (a) to (e) in the proportions set out in Table 3A.

TABLE 3A

Preparation No.	Proportion (Volume %)					
	CIT	ATS	ANS	NEEM	ALC	Water
3-1	1.6	66.4	-	16	16	-
3-2	44	44	-	6	6	-
3-3	-	88	-	6	6	-
3-4	-	88	-	6	6	-
3-5	-	98.4	-	0.8	0.8	-
3-6	-	-	-	6	6	88
3-7	-	-	-	0.8	0.8	98.4
3-8	-	100	-	-	-	-

The preparations were used to treat respective groups of three greyhound dogs. Each treatment consisted of

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a single application of the preparation by spraying 40-50ml on each dog and rubbing this into the dog's skin and fur. Some kennels were sprayed with 40ml per square metre of the respective preparation.

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Table 3B shows the mean number of fleas counted on each group before treatment (Day 0), and at 2, 12 and 20 days after treatment. The percentage increase or decrease in the number of fleas is given in parentheses, and the number of kennels treated in each group is given. In the untreated control group, one kennel was sprayed with 40ml per square metre of a solution made by dissolving 30ml of the neem extract in 1 litre of water.

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TABLE 3B

Preparation No.	Kennels Treated	Mean No. of Fleas			
		Day 0	Day 2	Day 12	Day 20
3-1	2	30	2(-93%)	6(-80%)	4(-87%)
3-2	2	35	1(-97%)	4(-89%)	12(-66%)
3-3	3	15	0(-100%)	12(-20%)	21(+40%)
3-4	0	22	2(-91%)	23(+4%)	20(-9%)
3-5	1	13	1(-92%)	15(+15%)	12(-7%)
3-6	2	20	9(-55%)	15(-25%)	13(-35%)
3-7	1	17	3(-82%)	5(-71%)	16(-6%)
3-8	1	17	1(-94%)	13(-23%)	21(+24%)
Untreated	-	24	14(-42%)	10(-76%)	15(-12%)

As can be seen from Table 3B, with the exception of the control group each of the treatments achieved a substantial reduction in the flea population after 2 days. However, only preparations 3-1 and 3-2 according to the present invention resulted in a reduction of over 50% after 20 days. From a comparison of the results for preparation 3-1 with those for preparations 3-3 and 3-4, it is apparent that only a small amount of oil of citronella is required to show a synergistic effect with DEET and azadirachtin.

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Comparative Example 4

The following preparations were made up:

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TABLE 4A

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Preparation No.	Proportion (Volume %)				
	CIT	ATS	ANS	NEEM	ALC
4-1	33.3	33.3	-	16.7	16.7
4-2	100	-	-	-	-
4-3	-	100	-	-	-
4-4	33.3	-	-	33.3	33.3

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Four groups of greyhound dogs were sprayed with an initial treatment of 40-50ml of each of the preparations, and thereafter sprayed with 5-10ml of each preparation at 3, 10, 28, 48 and 80 days after the initial treatment. The number of fleas on each group of dogs was recorded before the initial treatment (Day 0), and at 3, 10, 17, 28, 48, 80 and 120 days after the initial treatment, and the results are set out in Table 4B.

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TABLE 4B

Preparation No.	Mean No. of Fleas							
	Days							
	0	3	10	17	28	48	80	120
Untreated	42	65	73	46	34	42	12	32
- 4-1	56	0	2	4	12	5	2	8
4-2	64	34	53	23	45	33	32	61
4-3	86	98	65	38	39	65	67	98
4-4	73	21	24	43	23	21	39	49

It is apparent from Table 4B that only preparation 4-1 according to the present invention provided substantial long-term protection against fleas.

In a further aspect of the invention, it has been found that the application of neem extracts containing azadirachtin to the skin and coat of animals results in increased hair growth and reduced thickness of inflamed and eczematous patches of skin.

The results on Table 5 show the effect of one application of 50ml of alcoholic neem tree extract containing 6% azadirachtin on the mean rate of hair growth (mm/week) on greyhound dogs. The standard deviation for each mean is shown in parentheses.

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TABLE 5

Treatment	Dogs (Number)	Mean Hair Growth (mm/week)
Neem extract (15ml) in 35ml ethyl alcohol	6	2.34 (0.48)
Untreated	6	1.54 (0.43)
Ethyl alcohol (50ml)	6	1.67 (0.64)
Water (50ml)	6	1.82 (0.32)

The results on Table 6 show the effect of one application of 50ml of alcoholic neem tree extract containing 6% azadirachtin on the mean weight (Kg) of hair combed off greyhound dogs. The standard deviation for each mean is shown in parentheses.

TABLE 6

Treatment	Dogs (Number)	Hair weight Mean (grams)
Neem extract (15ml) in 35ml of ethyl alcohol	6	3.16 (0.21)
Untreated	6	1.54 (0.29)
Ethyl alcohol (50ml)	6	2.07 (0.32)
Water (50ml)	6	1.29 (0.82)

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The results on Table 7 show the effect of one application of 50ml of alcoholic neem tree extract containing 6% azadirachtin on the mean thickness of inflammed and eczematous skin patches of greyhound dogs after 14 days. The standard deviation of each mean is shown in parentheses.

TABLE 7

Treatment —	Dogs (No)	Mean Skin Thickness (mm)	
		Before Treatment	After Treatment
Neem extract (15ml) in 35ml of ethyl alcohol	4	5.64(0.13)	4.10(0.07)
Untreated	4	5.45(0.12)	5.56(0.19)
Ethyl alcohol (50ml)	5	5.33(0.23)	5.34(0.18)
Water (50ml)	4	5.01(0.21)	5.11(0.17)

It is not yet clear which of the components of the oil of citronella causes the synergy with DEET and azadirachtin. Preliminary results from further experimental work suggest that only small amounts of oil of citronella are required for the synergy to be displayed.

Although the present invention has been described with particular reference to the synergy between azadirachtin, oil of citronella and DEET, it is believed that the synergy will be effective with other

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5 dialkyltoluamides which may optionally be substituted  
with moieties which do not adversely affect the  
synergy, such as halogen, C<sub>1</sub>-C<sub>4</sub> alkyl and nitro  
groups. Similarly, it is believed that other oils or  
10 compositions containing geraniol, citronellal and/or  
other components of oil of citronella will also  
exhibit a synergy with azadirachtin and DEET or other  
dialkyltoluamides, and such combinations displaying  
synergy are intended to be embraced within the scope  
15 of the present invention.

The pesticidal and insect repellent compositions  
according to this invention display low toxicity to  
animals and birds and are suitable for direct  
15 application to the skin, coat or feathers of the  
animal or bird to prevent infestation by pests such  
as insects and arachnids.



CLAIMS

1. A pesticidal composition comprising azadirachtin, dialkyltoluamide and an oil containing geraniol and/or citronellal.
2. A pesticidal composition according to claim 1 wherein the alkyl moieties of the dialkyltoluamide are independently selected from C<sub>1</sub> to C<sub>4</sub>-alkyl.
3. A pesticidal composition according to claim 2 wherein the alkyl moieties are both ethyl groups.
4. A pesticidal composition according to claim 3 wherein the dialkyltoluamide is N,N-diethyl-m-toluamide.
5. A pesticidal composition according to claim 1 wherein the oil is oil of citronella.
6. A pesticidal composition according to claim 5 comprising from 1 to 35% by volume oil of citronella, from 30 to 200g per litre N,N-diethyl-m-toluamide and from 2 to 30g per litre azadirachtin in an inert carrier.
7. A pesticidal composition according to claim 6 wherein the carrier is non-aqueous.
8. A pesticidal composition according to claim 7 wherein the carrier comprises ethanol.
9. A pesticidal composition according to claim 1 further comprising di-n-propyl isochinchomerate and/or N-octyl bicycloheptene.

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10. A method of killing or repelling pests comprising applying dialkyltoluamide, azadirachtin and an oil containing genaniol and/or citronellal to the pests or their surroundings.
11. A method of killing or repelling pests on an animal or bird comprising applying dialkyltoluamide, azadirachtin and an oil containing geraniol and/or citronellal to the skin, coat or feathers of the animal or bird.
12. A method according to claim 10 or 11 wherein the dialkyltoluamide is N,N-diethyl-m-toluamide.
13. A method according to claim 10 or 11 wherein the oil is oil of citronella.
14. A method according to any of claims 10 or 11 wherein the pests are insects or arachnids.
15. A method of promoting hair growth on an animal comprising applying azadirachtin to the coat or skin of the animal.
16. A method of treating inflamed or eczematous skin on an animal comprising applying azadirachtin to the inflamed or eczematous skin.
17. A method according to claim 15 or 16 wherein the azadirachtin is applied as a solution of azadirachtin in ethanol.

**SUBSTITUTE SHEET**

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 90/00586

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.<sup>5</sup> A01N 65/00, 35/02, 37/18, 43/22, 31/02, A61K 7/06, 7/48, 31/35

## II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System |

Classification Symbols

IPC

A01N 35/02, 31/02, 37/18, 43/22, A61K 31/35, 7/06, 7/48  
KEYWORD: AZADIRACTIN OR NEEMDocumentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched 8

AU : IPC as above

## III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
A	AU-A-75714/87 (GUERRINI V.H.) 6 October 1988 (06.10.88) see pages 7-8.	1-14
A	AU-A-11220/88 (GUERRINI V.H.) 19 January 1989 (19.01.89) see pages 5-7.	1-14
A	AU-A-11221/88 (GUERRINI V.H.) 20 April 1989 (20.04.89) see page 5.	1-14
A	AU-A-26320/88 (UNIVERSITY OF QUEENSLAND: VETSEARCH AUSTRALIA PTY. LTD) 1 June 1989 (01.06.89) see claims 1 and 6.	1-14
(continued)		

\* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"G" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the  
International Search  
27 February 1991 (27.02.91)Date of Mailing of this International  
Search Report

9 April 1991

International Searching Authority

Signature of Authorized Officer

Australian Patent Office

S. CHEW



## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
T,A	AU-A-60185/90 (GUERRINI V.H.) 7 February 1991 (07.02.91) see page 2.	1-14
A	Journal of Insect Physiology, vol. 34, no. 7, pg. 713-719, 1988, Schmutterer H, "Potential of Azadirachtin-containing Pesticides for Integrated Pest Control in Developing and Industrialised Countries", see whole document.	1-14
X	GB-A-2000971 (AMINA LATIF) 24 January 1979 (24.01.79), see page 1 lines 11-14, 21-23.	15
P,X	Canadian Journal of Botany, vol. 68, no. 1, 1990, page 1-11. Koul. O et al "Properties and Uses of neem, Azadirachta indica", see page 4 col 1 paragraph 4.	16
A	DE,A, 3809427 (HEGELICH K) 5 October 1989 (05.10.89) see claim 1.	16-17

## FURTHER INFORMATION CONTAINED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

Claim 1 claims a composition comprising azadirachtin, dialkyltoluamide and oil (having geraniol and/or citronellal) whilst claims 15 and 16 claim the use of azadirachtin for hair growth in animals and treatment of exzematous skin on animals respectively. The only common feature between the claims ie: azadirachtin is not novel and thus the claims 15 & 16 each claims a different invention to claim 1.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☒ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2)) (January 1985)

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